## REMARKS

Claims 2-13, 16, and 19-23 are currently pending in the application. In order to advance prosecution, Applicant has amended claim 21. A complete listing of all the claims, in compliance with the revised amendment format, is shown above.

The amendments to the pending claims are made without prejudice, do not constitute amendments to overcome any prior art rejections under U.S.C. § 102 or 103, and are fully supported by the specification as filed. Support for all of the claim amendments can be found throughout the specification.

**Discussion of the 35 U.S.C. § 112, ¶ 2 Rejection**

Claims 2-13, 16, and 19-23 are rejected under 35 U.S.C. § 112, second paragraph, for being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. The rejection is overcome by amendment as follows.

With respect to claim 21, step b), the Office Action stated that the claim is vague and indefinite because it is unclear as to whether an average optical density of all stained target protein per pixel of cellular area in all the stained plurality of cells is determined, or that an average optical density of stained target protein pixel of cellular area for each of the stained plurality of cells, is determined. Although not acquiescing to this ground of rejection, Applicant has amended the claim to better clarify the invention. Applicant respectfully contends that the claim amendment has overcome the asserted ground of rejection.

With respect to claim 21, step c), the Office Action stated that the claim is vague and indefinite because it is unclear as to whether a calibration curve relating the known quantity of target protein with the average optical density of all stained target protein per pixel of cellular

area for all the stained plurality of cells, is generated, or that a calibration curve relating each known quantity of target protein with the average optical density of each stained target protein per pixel of cellular area for each of the stained plurality of cells, is generated. Although not acquiescing to this ground of rejection, Applicant has amended the claim to better clarify the invention. Applicant respectfully contends that the claim amendment has overcome the asserted ground of rejection.

Applicant respectfully contends that the outstanding grounds of rejection of the pending claims under 35 U.S.C. § 112, ¶ 2 have been traversed. In view of the above, Applicant respectfully requests withdrawal of all 35 U.S.C. § 112, ¶ 2 rejections.

**Discussion of the 35 U.S.C. § 103(a) Rejections**

Claims 2-11, 13, 16, and 19-23 are (presumably) rejected under 35 U.S.C. § 103(a) as being obvious over Slamon *et al* (U.S. Patent No. 5,846,749) ("Slamon") in view of Veltri et al. (U.S. Patent No. 6,463,438) (Veltri). Applicants note that the Office Action actually rejected these claims "under 35 U.S.C. 102(b) as being inherently anticipated by Slamon *et al.* (US Patent 5,846,749) in view of Veltri *et al.* (US Patent 6,464,438)." However, the Office Action acknowledges that Slamon differs from the instant invention in failing to disclose determining the optical density of stained target proteins per pixel of cellular area, and cites Veltri as a reference that allegedly renders the present invention obvious when combined with Slamon. Therefore, because a claim can only be rejected under 35 U.S.C. § 102 if each and every element as set forth in the claim is found in a single art reference, M.P.E.P. § 2131, and the Office Action refers to the two cited references as rendering the present invention obvious, Applicant presumes that the Office Action intended to reject these claims under 35 U.S.C. § 103(a). Clarification is

respectfully requested; however, Applicants respectfully traverse this presumptive ground of rejection with the following arguments.

An analysis for obviousness requires a determination of the scope and content of the prior art, the differences between the prior art and the claims at issue must be ascertained, and the level of ordinary skill in the pertinent art must be resolved. *Graham v. John Deere Co.*, 383 U.S. 1, 17 (1966). To establish a *prima facie* case of obviousness, the Office must show three basic criteria: (1) there must be a suggestion or motivation to combine the reference teachings; (2) there must be a reasonable expectation of success; and (3) all of the claimed limitations must be taught or suggested in the combined prior art references. M.P.E.P. § 2143.

The instantly claimed invention is directed towards methods for determining the quantity of a target protein in cells of a biological sample. This method requires, among other things, that the average optical density of stained target protein per pixel of cellular area be determined by, for example, image analysis. Because the average optical density of stained protein per pixel of cellular area is detected, the actual cells need never be identified and the number of cells present in the image field need never be actually determined. This determination is followed by either generating a calibration curve relating the known quantity of said target protein with the average optical density of stained target protein per pixel of cellular area, or using such a calibration curve to determine the quantity of a target protein in a biological sample.

None of the cited references, alone or in combination, teach or suggest the instantly claimed method. As acknowledged by the Office Action, Slamon does not teach a method of determining an "average optical density of stained target protein per pixel of cellular area." Instead, Slamon quantitates surface membrane and cytosolic proteins by determining the signal value from a *known* number of cells, and relates this value to values obtained with control cells.

Thus, according to the Slamon reference the signal value *per cell* is determined, rather than the average optical density of stained target protein *per pixel* of cellular area. As an example, all of the independent method claims of the Slamon reference require "determining the signal value *from a known number of said fixed cells* by computerized image analysis." *See, e.g., Slamon,* column 16, lines 12-14 (emphasis added). As a further example, the specification provides for measurement of protein immunostaining, specifically HER-2/*neu*, and measurement of DNA content in individual tumor cells. *See, e.g., id.* at column 10, lines 52-57. The Slamon reference does not, however, teach how to quantitate cellular proteins using image analysis *without* knowing or determining the number of cells that are immunostained. Moreover, the Slamon reference does not teach how to determine the "average optical density of stained target protein per pixel of cellular area," let alone how to use that information to determine the quantity of a target protein in cells of a biological sample. As a consequence, Slamon does not teach every limitation of the present invention.

The deficiencies of Slamon are not overcome by combination with the Veltri reference. Veltri describes a neural network used in a system to detect abnormalities in cells. *See Veltri,* Abstract. Veltri discloses a system "for self-adaptively and robustly distinguishing normal from abnormal tissue cells." *Id.* at column 3, lines 47-49. Further, "a neural network is provided which detects cancerous cells by analyzing raw images of the cell and providing the imaging information derived from the pixels of the images to a neural network." *Id.* at column 2, lines 61-65. In addition, "a neural network is provided which performs recognition of cancerous cells using information derived form an image of the cells, among other, the area, the average intensity, the shape, the texture, and the DNA content (pgDNA) of the cells." *Id.* at column 3, line 66 to column 4, line 4. Veltri even described the system disclosed as an advance to automate

bladder cancer cell detection, because the visual "inspection of thousands of cells" required in conventional staining techniques was labor intensive, and "the tedium and fatigue imposed upon the technician and the cytopathologist result in a high false negative rate" for the conventional techniques. *Id.* at column 1, lines 30-58.

The Office Action cites Veltri for the proposition that it teaches immunostaining of biomarker proteins in cells using biomarker specific antibodies to permit analysis of cellular, *i.e.*, nuclear, features. The Office Action further cites Veltri for the proposition that it discloses determining optical density of stained target protein per pixel of cellular area. However, Veltri does not teach a method of determining an "average optical density of stained target protein per pixel of cellular area," followed by either "generating a calibration curve relating the known quantity of said target protein with said average optical density of stained target protein per pixel of cellular area," or using such a calibration curve to determine the quantity of a target protein in a biological sample. Although Veltri teaches the necessity of using the optical density for the disclosed method, because the thickness of the cells can vary, and the light transmitted through any individual pixel falls off exponentially as a function of the object thickness (*id.* at column 10, lines 54-62), and further teaches immunostaining of biomarker proteins in the cells, the immunostaining as taught by Veltri is carried out in conjunction with Fuelgen staining (a DNA staining technique) to determine the various features of the individual cells, as described above, such as the area of the cell, the average intensity of staining of the cell, the shape of the cell, the texture of the cell, and the DNA content (pgDNA) of the cell. Contrary to the assertions in the Office Action, Veltri does not teach determining an average optical density of stained target protein per pixel of cellular area without knowing or determining the individual cells that are being analyzed. Further, Veltri certainly does not teach, much less suggest using the average

optical density of stained target protein per pixel of cellular area to determine the quantity of a target protein in cells of a biological sample. Therefore, Veltri does not itself teach, nor does it suggest, the presently claimed invention, nor does it supplement and overcome the deficiencies of the Slamon reference set forth above.

In addition, due to the deficiencies of both of the cited references Applicants respectfully contend that the Office Action has failed to establish a *prima facie* case of obviousness because there is no teaching, suggestion or motivation to combine the cited references, nor would the combination produce the invention even if made. The Office Action baldly argues that it would have been obvious to incorporate the teaching of Veltri in determining optical density of stained target protein per pixel of cell areas into the method of Slamon without providing any support for such an assertion, and thus fails to establish *prima facie* obviousness of the pending claims in view of the cited references.

Moreover, the mere description in Slamon of the use of image analysis to quantitate a protein such as HER-2/*neu* by determining the signal value for a *known* number of cells does not amount to a teaching or suggestion to determine the average optical density of stained target protein per pixel of cellular area, much less how to use this information in the claimed methods for determining the quantity of target protein in cells of a biological sample. Veltri does not cure this infirmity. Veltri is directed to describing a neural network used in a system to detect abnormalities in cells, using information derived from an image of cells, such as the area of the cells, the average intensity of the cells, the shape of the cells, the texture of the cells, and the DNA content (pgDNA) of the cells. Neither of these references contemplate or teach methods for determining the average optical density of stained target protein per pixel of cellular area, followed by either generating a calibration curve relating the known quantity of said target

protein with said average optical density of stained target protein per pixel of cellular area, or using such a calibration curve to determine the quantity of a target protein in a biological sample. *A fortiori*, the references certainly do not teach or even contemplate the instantly-claimed methods of determining the quantity of a target protein in cells of a biological sample. In the absence of such teaching, Applicants contend there was simply no motivation to combine these references as the Office Action suggests.

Applicants respectfully submit that the Office Action has engaged in impermissible hindsight to support its argument. In this regard, the Federal Circuit dictates, "[i]t is impermissible to use the claimed invention as an instruction manual or 'template' to piece together the teachings of the prior art so that the claimed invention is rendered obvious. This court has previously stated that '[o]ne cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention.'" *In re Fritch*, 23 U.S.P.Q.2d 1780, 1784 (Fed Cir. 1992) (citations omitted) (quoting *In re Fine*, 5 U.S.P.Q.2d 1596, 1600 (Fed. Cir. 1988)). Applicants respectfully submit that what the law precludes is precisely the basis for the asserted obviousness rejection.

For the reasons set forth above, neither Slamon nor Veltri, cited in support of this ground of rejection, taken either alone or in combination, disclose, suggest or motivate the skilled worker, either individually or in combination, to a method for determining the quantity of target protein in cells of a biological sample by, *inter alia*, determining the average optical density of stained target protein per pixel of cellular area, followed by either generating a calibration curve relating the known quantity of said target protein with the average optical density of stained target protein per pixel of cellular area, or using such a calibration curve to determine the

quantity of a target protein in a biological sample. Accordingly, Applicants respectfully request withdrawal of this rejection and requests reconsideration of the claims.

Claim 12 is rejected under 35 U.S.C. 103(a) as being unpatentable over Slamon in view of Veltri as applied to claims 2-11, 13, 16, and 20-23, and further in view of McNamara et al. (U.S. Patent 6,007,996) ("McNamara"). Applicants respectfully traverse this ground of rejection.

The instantly claimed invention is directed to a method for determining the quantity of a target protein in cells of a biological sample. As stated above, this method requires determining the average optical density of stained target protein per pixel of cellular area, followed by either generating a calibration curve relating the known quantity of said target protein with the average optical density of stained target protein per pixel of cellular area, or using such a calibration curve to determine the quantity of a target protein in a biological sample. In addition, as the Office Action notes, claim 12 requires, among other things, that the biological sample be stained with a multiplicity of stains.

None of the cited references, either alone or in combination, teach the claimed invention. The teachings and deficiencies, as related to the present invention, of Slamon and Veltri are discussed above, and apply with equal force to this ground of rejection. The Office Action acknowledges that Slamon and Veltri differ from claim 12 in failing to disclose staining with multiplicity of stains the biological sample upon which image analysis is performed. The deficiencies of Slamon and Veltri are not overcome by combination with McNamara. McNamara discloses, among other things, a method of *in situ* analysis of a biological sample comprising the steps of staining the biological sample with at least three stains, and collecting spectral data from the stained biological sample, where the spectral data device can collect data

from all the stains. *See McNamara*, column 55, line 66-column 56, line 24. McNamara does not provide any teaching whatsoever related to determining the average optical density of stained target protein per pixel of cellular area, nor generating a calibration curve relating the known quantity of said target protein with the average optical density of stained target protein per pixel of cellular area, or using such a calibration curve to determine the quantity of a target protein in a biological sample. Therefore McNamara does not render claim 12 obvious when combined with the Slamon and Veltri references, and none of the cited art provides the required teaching, suggestion or motivation that the McNamara reference be combined with Slamon and Veltri to arrive at the present invention.

Applicant respectfully contends that rejection on 35 U.S.C. § 103 grounds has been traversed by their argument herein, and request that this rejection be withdrawn.

**Conclusion**

In view of the above remarks, the application is considered to be in good and proper form for allowance and the Examiner is respectfully requested to pass this application to issue. If, in the opinion of the Examiner, a telephone call would expedite the prosecution of this application, the Examiner is invited to call the undersigned attorney.

Respectfully Submitted,

Date: June 16, 2004

Andrew W. Williams, Ph.D.
Reg. No. 48,644

McDonnell Boehnen Hulbert & Berghoff LLP
300 South Wacker Drive
Chicago, IL 60606
(312) 913-0001